Chapter 2

Mendelian Disorders of High-Density Lipoprotein Metabolism

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ABSTRACT

High-density lipoproteins (HDLs) are a highly heterogeneous and dynamic group of the smallest and densest lipoproteins present in the circulation. This review provides the current molecular insight into HDL metabolism led by articles describing mutations in genes that have a large effect on HDL cholesterol levels through their roles in HDL and triglyceride metabolism. Using this information from both human and animal studies, it is discussed how HDL is produced, remodelled in the circulation, affected by factors that control the metabolism of triglyceride-rich lipoproteins, how it helps maintain cellular cholesterol homeostasis, and, finally, how it is catabolized. It can be concluded that HDL cholesterol as a trait is genetically heterogeneous, with as many as 40 genes involved. In most cases, only heterozygotes of gene variants are known, and HDL cholesterol as a trait is inherited in an autosomal-dominant manner. Only 3 Mendelian disorders of HDL metabolism are currently known, which are inherited in an autosomal recessive mode.

NONSTANDARD ABBREVIATIONS AND ACRONYMS

ABCA1, G1 ATP-binding cassette transporter A1, G1
ANGPTL3 angiopoietin-like 3
apoA-I apolipoprotein AI
apoM apolipoprotein M
CAD coronary artery disease
CE cholesteryl ester
CETP cholesteryl ester transfer protein
CVD cardiovascular disease
FC free cholesterol
GWAS genome-wide association study
HDL high-density lipoprotein
HDL-C high-density lipoprotein cholesterol
LCAT lecithin:cholesterol acyltransferase
L**DL-C** low-density lipoprotein cholesterol
LIPC hepatic lipase
LIPG endothelial lipase
LPL lipoprotein lipase
ORPs oxysterol-binding protein–related proteins
PLTP phospholipid transfer protein
SNP single nucleotide polymorphism

sPLA2 secreted phospholipase A2
SR-B1 scavenger receptor class B member 1
TRIB1 tribbles homolog 1
TRL triglyceride-rich lipoprotein
VLDL very-low-density lipoprotein

INTRODUCTION

Mendelian disorders refer to diseases caused by mutations in single genes that are inherited following a simple pattern. When considering high-density lipoprotein (HDL) metabolism, 3 such disorders can be distinguished. These are APOAI, LCAT, and ABCA1 (encoding apolipoprotein AI [apoA-I], lecithin:cholesterol acyltransferase [LCAT], and ATP-binding cassette transporter A1 [ABCA1], respectively) deficiency, which all cause a loss of the capacity to produce or mature HDL. All 3 are inherited in an autosomal-recessive manner. Although homozygotes and compound heterozygotes for loss-of-function mutations in these genes mostly have clinical complications,[1] heterozygotes are generally without clinical symptoms. In this context, HDL cholesterol (HDL-C) levels can be considered a Mendelian trait with levels depending on the action of single gene products. On the contrary, this trait is genetically also heterogeneous because >40 different genes are currently reported to affect HDL-C levels. In the majority of cases, only heterozygotes for loss-of-function mutations are known and, despite their effect on HDL-C levels, they are again not reported to cause disease. This review is an attempt to discuss the genes for which there exists clear evidence that they play important roles in regulating HDL-C levels in humans and mice. The Figure gives an overview of the knowledge that has been obtained through mutations (or targeted disruption) of these genes and illustrates the current molecular details of HDL anabolism, conversion, and catabolism. The Table summarizes how mutations in these genes may affect atherosclerosis.

Terminology

Definitions of HDL and HDL-C

The mobilization of cellular cholesterol by HDL, the transport of cholesterol by HDL in the circulation, and the hepatic up- take of HDL-C are generally considered as key to the function of this lipoprotein class. However, from a biochemical perspective, HDL-C is a mere figure indicating how much

cholesterol is carried by the pool of HDL in blood. Unfortunately, the terms HDL and HDL-C are often used interchangeably. This leads to confusion and sometimes wrong interpretations. In the lay literature, HDL-C is often typed as the good cholesterol, suggesting that cholesterol is beneficial as long as it is in HDL that diverts from the multiple functions that are attributed to HDL that has little if nothing to do with its cholesterol component. In this review, HDL is used when either the particle or the pool of circulating HDL is meant, whereas HDL-C refers to the free cholesterol (FC) and cholesteryl ester (CE) carried by HDL in the circulation. HDL deficiency indicates that HDL and, therefore, HDL-C are (close to) absent. In an attempt to be comprehensive, we have combined genetic insights from both animal and human studies. Finally, the terms hypoalphalipoproteinemia and hyperalphalipoproteinemia are used when HDL-C concentrations are <10th percentile or >90th percentile for age and sex, respectively.

Reverse Cholesterol Transport and Cellular Cholesterol Homeostasis

Reverse cholesterol transport is generally used to describe the transport of cholesterol by HDL from the vascular wall to the liver for excretion into bile as neutral sterol or bile acid. Despite ≈50 years of research, there is, as of yet, little evidence that HDL can transport cholesterol from the vessel wall to the liver for catabolism. The overall key scheme presented in the Figure is thus not meant to illustrate the routing of cholesterol that may be mediated through HDL but rather to illustrate the main HDL pathways known to date.

Structure and Composition of HDL

HDLs are characterized by several distinct subpopulations. By ultracentrifugation, one can distinguish 2 main subfractions, namely the larger HDL_2 and the smaller and denser HDL_3. The dynamic macromolecular HDL complexes range from 70 to 100 Å in diameter and from 200,000 to 400,000 daltons in mass, are rich in protein (50%), and transport tri- glycerides and CE packaged in a monolayer of phospholipids and apolipoproteins.[1] ApoA-I and apoA-II are the major structural components of HDL, and many other amphipathic apolipoproteins[104] are isolated in HDL preparations. Adding to the complexity, many HDL-associated bioactive lipid species are thought to play important roles in various processes.

Main Determinants of HDL-C Levels

Genes

Family and twin studies have shown that circulating levels of HDL-C have a strong inherited basis, with heritability estimates ranging from 40% to 80%.[105–107] Accordingly, numerous genes affecting HDL metabolism have been described in humans or mice or both. Recently, data have emerged that suggest that extreme levels of HDL-C in families can have a polygenic origin,[59,108,109] and that common genetic variation can explain a large proportion to the heritability of HDL-C levels.[110]

One can generally distinguish between genes that directly affect de novo HDL genesis and those affecting HDL more indirectly through, for example, affecting hepatic triglyceride output or affecting the lipolysis of triglyceride-rich lipoproteins (TRL). This latter process likely explains the generally tight inverse relationship between plasma levels of HDL-C and triglycerides. Increased plasma triglyceride lipolysis can increase HDL-C levels[111] but, on the contrary, this process cannot recapitulate HDL-C levels in case the de novo production of HDL is completely disrupted as, for example, in APO-AI deficiency.

Lifestyle and Disease States

Age and sex belong to the no modifiable risk factors influencing plasma HDL-C levels, with age being positively correlated with HDL-C levels[112] and male sex being associated with lower HDL-C.[113] On the contrary, obesity, diet, physical activity, smoking, alcohol, and drugs are part of modifiable risk factors.[107,114]

It is furthermore well-acknowledged that disease-related states, such as type 2 diabetes mellitus, metabolic syndrome, and kidney disorders, are all associated with reduced HDL-C levels. In addition to an increased turnover and remodelling of large HDL, these conditions all feature dense and small HDL, suggestive of an impaired conversion from small to large HDL.[115,116] Alcohol intake increases HDL-C in a dose-dependent fashion,[117] whereas smoking is associated with low circulating levels of HDL-C,[118] and several classes of drugs affect HDL metabolism.[28] Recent studies have also shown that HDL-C levels are low in patients with liver failure and even reflect its severity.[119] Low plasma HDL-C levels are associated not only with an increased risk of cardiovascular disease (CVD) but also with the rate and incidence of cancer[120] as well as neurological disorders.[121] Combined, the data suggest that

HDL-C levels can be considered as a general biomarker for compromised health. Thus, plasma HDL-C levels are an outcome measure of genetics, lifestyle, and possible disease states.

HDL and CVD

Epidemiology and Pharmaceutical Modulation

Epidemiological studies have indisputably shown that low circulating levels of HDL-C represent a significant, robust, and independent predictor of CVD.[122,123] This association was first reported by Barr et al[124] and was ignored until attention was given by the Framingham Heart Study. HDL-C has since been used as an important risk factor to assess cardiovascular risk. In light of these observational findings, clinical trials have been performed to study whether intervention to increase HDL-C would result in reduced risk of CVD[125,126] but, so far, no trials have proved to be effective. Details of these studies and those that are ongoing are discussed elsewhere in this review series.

Complete Loss of Function of Major HDL Genes in Humans

The number of reported families with severe HDL disorders is small and, as a consequence, it is hazardous to speculate on the risk of CVD. In the Table, we have tried summarizing the current data. Most of the mutations in *APOAI* are associated with increased CVD.[127–129] However, heterozygotes of the apoA-I Milano variant exhibit low HDL-C levels but reduced premature coronary artery disease (CAD),[130,131] whereas carriers of the apoA-I Paris variant have also been reported to be protected against CAD onset.[132] Carriers of mutations in *LCAT* that cause HDL deficiency and 40% reductions of HDL-C in homozygotes and heterozygotes, respectively, have been reported to be at increased risk and decreased risk of atherosclerosis.[133,134] Also, when it comes to HDL deficiency caused by mutations in ABCA1, evidence supporting an increased risk of CVD is unequivocal.[2,135,136] On the contrary, cholesteryl ester transfer protein (*CETP*) deficiency causes strong increases in HDL-C levels. Although genetic *CETP* deficiency was first considered to be associated with low morbidity from CAD and longevity,[137] this was subsequently the subject of debate.[138] To date, only a few patients with hepatic lipase (*LIPC*) deficiency (encoding LIPC) have been described,[139,140] whereas *LIPC* promoter variants

are associated with elevated plasma levels of HDL-C and paradoxically increased cardio- vascular risk.[141,142] Because most family studies originate from index patients who were referred to the clinic, one may ask the question whether mutations in any of these genes are associated with altered CVD risk in the general population.

Genetic Population Studies

Because this is a topic of another review in this series, we only briefly address this topic. There is evidence that both rare and common alleles with major phenotypic effects contribute significantly to low plasma HDL-C levels in the general population.[65,143,144] Recent whole-genome sequencing and analysis suggested that common variation contributes more to heritability of HDL-C levels than rare variation.[110] However, it has become clear that genetic variation causing either increased or decreased levels of HDL-C is generally not associated with the anticipated low risk and higher risk of atherosclerosis.[145,146] Also, results from genome-wide association studies (GWAS) have shown that variation in genes associated with HDL-C levels are not associated with CVD, whereas by contrast this is the case for variation in genes associated with low-density lipoprotein cholesterol (LDL-C) levels.[61]

Mendelian Randomization Studies

This approach has been recently used to investigate whether genetically altered HDL-C levels associate with the estimated risk of cardiovascular events: genetic information is used to test for associations between intermediate phenotypes, such as HDL-C levels, and disease outcome.[147] In 2 such studies, several single nucleotide polymorphisms (SNPs) consistently associated with high HDL-C levels were not found to be associated with cardiovascular events.[65,148] Clinical and genetic studies to date have shown that changes in HDL-C concentration are generally not associated with the anticipated outcome. Reconsidering these recent outcomes, many investigators point to the notion that the plasma level of HDL-C does not account for beneficial functions associated with HDL.[1,149] This is true, but none of the HDL function parameters or biomarkers have yet provided answers why increasing HDL-C did not provide the anticipated atheroprotection. It should also be kept in mind that epidemiological studies show that it is the level of HDL-C in plasma that has prospective value. Clearly, new tools and

approaches are needed to unravel how HDL and HDL-C relate to pathogenesis.[150]

HDL METABOLISM FROM A GENETIC PERSPECTIVE

The Figure illustrates the roles of most of the major genes involved in the genesis, conversion, and catabolism of HDL. We describe the genes involved in the biogenesis of the nascent HDL and its maturation. In the Table, we have summarized the main findings in both humans and mice.

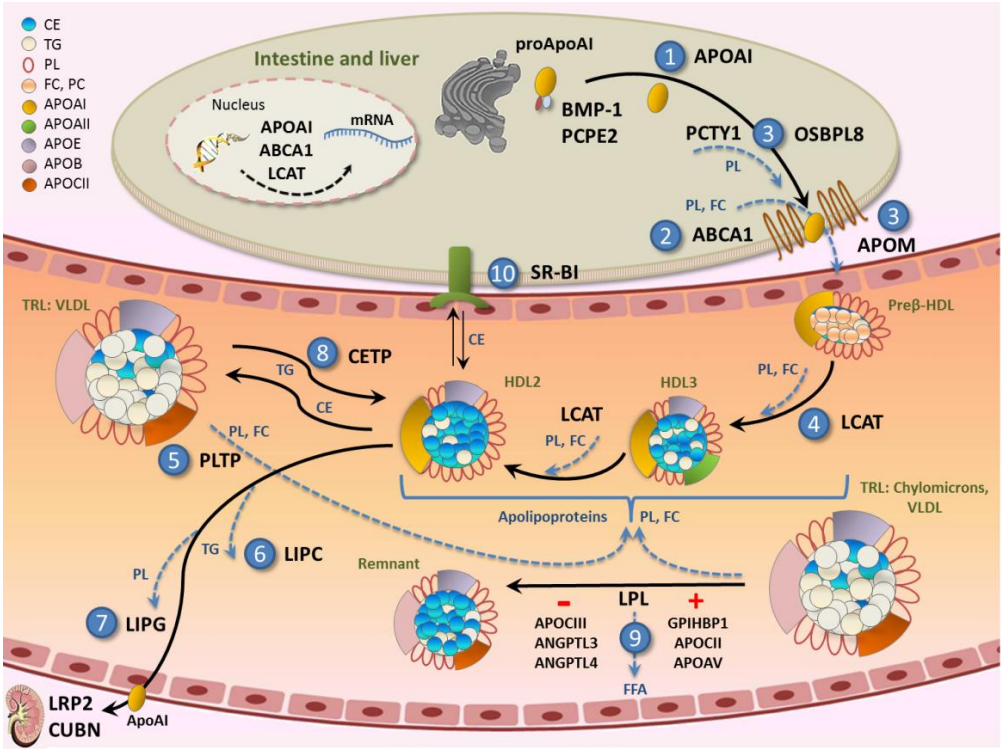

Figure 1. Illustration of the major genes involved in the genesis (1–4), remodelling (5–9), and catabolism (7,8) of high-density lipoprotein (HDL). The figure is based on data obtained through studies in humans and mice. **1**, The liver and small intestine are the only organs able to produce nascent HDL. This involves the intracellular maturation of proapolipoprotein AI (apoA-I) into apoA-I requiring bone morphogenetic protein-1 (BMP1) and procollagen C-

proteinase enhancer-2 protein (PCPE2), followed by (**2**) the early acquisition of phoshpholipids (PL) and free cholesterol (FC) of apoA-I through ATP binding cassette transporter A1 (ABCA1) activity, which results in the production of pre-β-HDL at the cellular membrane. In this process (**3**) CTP:phosphocholine cytidylyltransferase (PCYT1; delivery of PL) and also apolipoprotein M (ApoM) and oxysterol-binding protein-related protein 8 (OSBPL8) have been shown to play a role. In the circulation (**4**), nascent HDL matures through the acquisition of cholesteryl ester (CE) that are generated by lecithin:cholesterol acyltransferase (LCAT). The enrichment of the core of HDL with CE causes HDL to become spherical and less dense (generating the HDL_3 and HDL_2 subspecies). **5**, Cholesteryl ester transfer protein (CETP) facilitates transfer of CE from HDL to triglyceride-rich lipoprotein (TRL) in exchange for triglycerides (TG). **6**, Phospholipid transfer protein (PLTP) is known to fuse smaller HDL (not shown here) and to accommodate the transfer of PLs from TRL to HDL. The actions of both hepatic lipase (LIPC) and endothelial lipase (LIPG; **7**, **8**) in breaking down HDL-TG and PL, respectively, enhance the dissociation of lipid-free or lipid-poor apoA-I from larger HDL, making HDL prone to renal catabolism via low-density lipoprotein–related protein 2 (LRP2) and cubilin (CUBN), which are thought to play a role here. **9**, Many factors affect lipoprotein lipase (LPL)–mediated lipolysis of TG in triglyceride-rich lipoproteins (TRL), which include activators /modulators (apolipoprotein CII [APOCII], AV, GPI-anchored HDL binding protein-1 [GPIHBP1]) and inhibitors (APOCIII, ANGPTL3,4) of this reaction. The LPL reaction frees constituents (apolipoproteins, PL, and FC) for the pool of HDL particles. **10**, SR-BI, the main HDL receptor, can mediate the uptake of CE in the liver and in steroidogenic tissues (latter not shown here). PC indicates phosphatidylcholine.

Table 1. Genes With Established Functions in Human and Murine HDL Metabolism

Gene (Chr. Position)	Complete Gene Loss in Humans	No. of Mutations and Individuals		Coronary Artery Disease	Genetic Association Studies	Knockout and Silencing Studies	Transgenic Mice and Overexpression
ABCA1 (9q31.1)	Tangier disease, hypoalphalipoproteinemia	Total	175	Variable effect on atherosclerosis.[2,3]	Common variants are variably associated with HDL-C levels and CAD risk[4,5]	Tangier-like phenotype,[6,7] no reduced cholesterol excretion.[8] No increased atherosclerosis.[9] siRNA: reduction of HDL-C, HDL-associated ApoA-I, ApoE, in mice.[10]	Increased HDL-C; increased or decreased atherosclerosis.[11–13] Increased apoB levels and accelerated atherosclerosis on LDLr$^{-/-}$ background.[12] Decreased atherosclerosis following transplantation of bone marrow from ABCA1transgenics into LDLr$^{-/-}$ mice but no effects on lipid profile.[14]
		−/−	102				
		+/−	232				
		Comp +/−	36				
ABCG1 (21q22.3)	—	…		…	One variant associated with HDL-C levels.[15] Variants associated with reduced and increased CAD risk in absence of effects on plasma lipids.[16,17]	Cholesterol accumulation in tissues (lung); no effect on plasma lipoprotein levels.[18] Accelerated atherosclerosis in ABCA1$^{-/-}$[19] and LDLr$^{-/-}$.[20] siRNA: reduction of cholesterol and phospholipid efflux to HDL in murine macrophages.[21]	Protection from diet-induced cellular cholesterol accumulation.[18]
APOAI (11q23-q24)	apoA-I deficiency Hypoalphalipoproteinemia	Total	63	Increased risk[22,23]	Both rare and common variants are associated with low or high HDL-C levels.[24,25]	Normal HDL-C levels but increased atherosclerosis in mice lacking LDLr.[26]	Stimulation of macrophage-specific reverse cholesterol transport[27] and decreased atherosclerosis when crossed on different atherogenic backgrounds.[28,29]
		−/−	24				
		+/−	219				
		Comp+/−	6				
APOAV (11q23)	apoAV deficiency Hypertriglyceridemia Hypercholesterolemia Hypoalphalipoproteinemia	Total	38	Few reports with increased risk.[30,31]	Most common variants associated with decreased levels of HDL-C, increased levels of TG,[32–36] and increased CAD risk.[30,31]	Disruption of TRL metabolism.[37] Increased TG levels, no significant changes in plasma HDL-C levels.[38]	Decreased plasma TG levels but no significant changes in plasma HDL-C levels.[38]
		−/−	7				
		+/−	43				
		Comp+/−	1				
APOCII (19q13.2)	apoCII deficiency Chylomicronemia Hypertriglyceridemia Hypoalphalipoproteinemia	Total	18	A case-control study suggests an increased risk.[39]	Rare and common variants associated with high TG.[40,41] No data on HDL-C levels.	…	Marked hypertriglyceridemia with accumulation of triglyceride-enriched VLDL; HDL-C is minimally decreased.[42]
		−/−	30				
		+/−	26				
		Comp+/−	—				
APOCIII (11q23.3)	apoCIII deficiency Hyperalphalipoproteinemia	Total	12	One study showed reduced risk.[43]	Variants associated with high HDL-C levels and decreased atherosclerosis.[43]	Enhanced uptake of TG-derived free fatty acids by adipose tissue; no effect on the VLDL-TG production.[44] Hypotriglyceridemia and protection from postprandial hypertriglyceridemia.[45]	Increased plasma TG.[46,47] Increased risk of atherosclerosis because of enhanced endothelial dysfunction.[48]
		−/−	6				
		+/−	76				
		Comp+/−	—				
ApoM (6p21.33)	—	—		One study suggesting no effect.[49]	—	Reduced conversion of HDL to preβ-HDL on LDLr$^{-/-}$ background; knockdown leads to reduction of preβ-HDL in mice.[50]	Increased HDL-C and reduced atherogenesis.[50,51]
CETP (16q21)	CETP deficiency Hyperalphalipoproteinemia	Total	39	Pro- and antiatherogenic effects.[52,53]	Common genetic variation is associated with HDL-C and CAD risk.[54,55]	Mice are naturally CETP deficient. Increased apoA-I and HDL efflux, decreased HDL-uptake in HepG2 cells after inhibition via antisense oligodeoxynucleotides.[56]	Reduced HDL-C and apoA-I levels,[57] Variable atherosclerosis.[57,58]
		−/−	97				
		+/−	403				
		Comp+/−	32				

Table1. Continued

Gene (Chr. Position)	Complete Gene Loss in Humans	No. of Mutations and Individuals		Coronary Artery Disease	Genetic Association Studies	Knockout and Silencing Studies	Transgenic Mice and Overexpression
GALNT2 (1q41-q42)	—	—		—	Rare variants associated with increased HDL-C[59,60] Common variant affects HDL-C and TG concentration.[61]	Higher HDL-C levels following knockdown in mice.[61]	Reduced HDL-C levels.[61]
LCAT (16q22.1)	Familial LCAT deficiency Fish-eye disease Hypoalphalipoproteinemia	Total	94	Increased and decreased carotid intima-media thickness.[62–64]	One variant associated with increased HDL-C but not with risk of MI.[65]	HDL deficiency.[66] Increased atherosclerosis in LDLr$^{-/-}$ and apoE$^{-/-}$ mice.[67]	No effect on atherosclerosis in wild-type mice.[68] Increased HDL-C and atherosclerosis.[69] Both increased or reduced atherosclerosis on apoE$^{-/-}$ and LDLr$^{-/-}$ background.[67,70] Gene therapy decreases plaque volume in LDLr$^{-/-}$ and ob/ob mice.[71]
		−/−	72				
		+/−	100				
		Comp+/−	46				
LIPC (15q21-q23)	LIPC deficiency Hyperalphalipoproteinemia	Total	19	Effect on CAD unclear.[72]	Common variants associated with increased HDL-C and CAD risk.[73,74]	Increased HDL-C and reduced atherosclerosis in apoE$^{-/-}$ mice.[72]	Reduced HDL-C and apoAI levels[72] and atherosclerosis in double-knockout (LIPC/apoE) mice.[75]
		−/−	8				
		+/−	40				
		Comp+/−	13				
LIPG (18q21.1)	LIPG deficiency Hyperalphalipoproteinemia	Total	17	One case report of atheroprotection.[76]	Common variants associated with increased and decreased HDL-C.[59,77,78]	Increased HDL-C.[79] Reduced atherosclerosis in apoE$^{-/-}$ mice.[80] siRNA: decreased cholesterol, TG, and proinflammatory cytokine expression in THP-1 macrophages.[81]	Reduced HDL-C.[79,82]
		−/−	2				
		+/−	256				
		Comp+/−	1				
LPL (8p22)	LPL deficiency Hypertriglyceridemia Chylomicronemia Hypoalphalipoproteinemia	Total	161	Variable effect on atherosclerosis.[83,84]	Loss-of-function and gain-of-function variants associated with increased and decreased HDL-C, respectively.[32,85]	Reduced HDL-C levels.[86] siRNA: reduction of intracellular lipid levels in 3T3-L1 adipocytes,[87] increased free cholesterol.[88]	Increased HDL-C.[86]
		−/−	129				
		+/−	158				
		Comp+/−	61				
PLTP (20q12-q13.1)	—	…		One report of decreased risk.[89]	Common variants associated with increased number of HDL particles, smaller HDL size, and decreased CAD risk.[89]	Reduced HDL-C and ApoAI levels and increased atherosclerosis.[90,91]	Increased HDL/non-HDL cholesterol ratio.[92] Increased atherogenesis[93] on LDLr$^{-/-}$ background.[94]
SCARB1 (12q24.31)	—	—		—	Common variants associated with increased HDL-C but sex-dependent.[95–97]	High HDL-C[98]; increased atherosclerosis.[99] Severe atherosclerosis in apoE$^{-/-}$.[100] siRNA: increased cholesterol uptake and decreased cholesterol efflux in CaCo-2 cells.[101]	Decreased HDL-C, increased clearance of HDL and non-HDL cholesterol.[102] Reduced atherosclerosis in LDLr−/+.[103]

Individuals: −/−, homozygotes; −/+, heterozygotes; comp−/+, compound heterozygotes. Mutations retrieved from HGMD professional (Human Gene Mutation Database, last accessed March 2013). ApoA-I indicates apolipoprotein AI; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; Chr., chromosome; HDL-C, high-density lipoprotein cholesterol; HepG2 human liver hepatocellular carcinoma cell line; LCAT, lecithin:cholesterol acyltransferase; LDLr, low density lipoprotein receptor; LIPC, hepatic lipase; LIPG, endothelial lipase; LPL, lipoprotein lipase; MI, myocardial infarction; siRNA, small interfering RNA; TG, triglycerides; THP, human acute monocytic leukemia cell line; TRL, triglyceride-rich lipoprotein; VLDL, very-low density lipoprotein cholesterol.

Biogenesis of Nascent HDL and Its Early Maturation

Apolipoprotein AI

The de novo synthesis of HDL involves the secretion of apoA-I by the liver and small intestine into the circulation, followed by a largely extracellular acquisition of phospholipids (PL) and cholesterol leading to the formation of nascent HDL (**step 1** in Figure). The gene is located on chromosome 11q23 and encompasses 4 exons encoding a primary transcript of 267 amino acids. *APOAI/ApoAI* gene deletion results in extremely low levels of HDL-C in both humans and mice, respectively.[151] Intracellularly, an 18-amino-acid pre-peptide is cleaved at the endoplasmatic reticulum by a signal peptidase, whereas an intermediate pro–apoA-I with a hexa- peptide extension at its amino (N-) terminus[152] is secreted into extracellular fluids and plasma. Subsequent cleavage of the hexapeptide produces the mature 243-amino-acid protein, which is necessary for the assembly of small disc-shaped native HDL.[153] In vitro studies have shown that the latter reaction is mediated by bone morphogenetic protein-1.[154] Recent studies in knockout (KO) mice have also highlighted a key role for procollagen C-proteinase enhancer-2 protein in mediating this last step of apoA-I maturation. The data suggest the mandatory involvement of a ternary complex composed of pro–apoA-I, bone morphogenetic protein-1, and procollagen C-proteinase enhancer-2 protein.[155] Multiple other HDLs can be formed in the absence of apoA-I with roles for apoA-II, apoE, and apoA-IV,[156] but without apoA-I present HDL-C levels are generally low. To date, 63 mutations have been identified in *APOAI*[157] with >70% variants directly implicated in hypoalphalipoproteinemia. ApoA-I deficiency is a rare disorder that is characterized by the total absence of apoA-I in the circulation along with a low or absent HDL-C,[22,158] whereas LDL-C and tri- glyceride levels are not affected. Typical clinical symptoms of apoA-I–deficient patients are xanthomas and mild-to- moderate corneal opacification. Heterozygote individuals present with ≈50% of normal HDL-C and apoA-I levels without explicit clinical symptoms.[159] Although the impact of *APOAI* mutations is variable, they seem to cause the utmost elevation in cardiovascular risk compared with mutations in other genes implicated in HDL metabolism.[22,158,160]

ATP-Binding Cassette Transporter A1

The early apoA-I lipidation with FC and phosphatidylcholine (PC) occurs on its critical interaction with ABCA1 and results in the formation of discoidal pre–β-HDL particles (**step 2**). Its major role in HDL biogenesis was recognized after the discovery in 1999 that lack of ABCA1 (9q31.1) causes HDL deficiency in Tangier disease.[135,161,162] An impaired FC efflux from Tangier cells leads to intracellular accumulation of CE visible in the characteristic deposits of lipids in lymphoid organs, such as the tonsils, and can be accompanied by other clinical features including peripheral neuropathy, hepatosplenomegaly, corneal opacities, thrombocytopenia, premature myocardial infarction, or stroke.[135] This disorder has since been diagnosed in ≈100 patients worldwide,[163] and >170 mutations have been reported. In patients with Tangier disease, plasma levels of apoA-I are only 3% of that of controls, whereas triglyceride levels (>200 mg/dL) are increased along with reduced LDL-C (50% of normal). Heterozygotes for deleterious mutations present with half-normal levels of HDL-C and apoA-I but without apparent clinical symptoms.[164,165] When ABCA1 function is impaired, apoA-I cannot be lipidated, leading to its rapid clearance from the plasma circulation, resulting in significantly reduced levels of apoA-I and the presence of only small pre–β-HDL particles. The importance of ABCA1 to maintain normal HDL-C levels in mice was illustrated by liver-specific ABCA1 KO mice showing an 80% decrease in HDL-C levels.[166]

Apolipoprotein M

ApoM is an HDL-associated apolipoprotein that affects HDL biogenesis by affecting nascent pre–β-HDL assembly through ABCA1 (**step 3**).[167] Wolfrum et al[50] demonstrated that apoM KO mice have impaired HDL interconversion and store cholesterol in large HDL. ApoM deficiency decreases plasma HDL-C concentrations by ≈25%.[50] Furthermore, RNA-mediated knockdown of ApoM in vivo causes a reduction in pre–β-HDL.[50,51] It has been shown that variation in the promoter region of *ApoM* gene is associated with plasma cholesterol levels,[168] but this was not replicated.[169] Interestingly, Arkensteijn et al[170] recently showed a specific role of apoM as a carrier of the sphingosine 1 phosphate. This sphingholipid activates 5 different G-protein–coupled receptors that affect numerous vascular functions.[171,172] Recently, Karuna et al[173] reported that plasma levels of sphingosine 1 phosphate in apoM KO and transgenic mice were reduced by 30% and increased by 270%, respectively. In

addition, mutations in *APOA1*, *ABCA1,* or *LCAT* in humans reduced plasma levels of HDL-C and apoA-I as well as sphingosine 1 phosphate in an apparent gene–dose-dependent fashion. In contrast, mutations that increase plasma concentrations of both HDL-C and apoA-I did not affect sphingosine 1 phosphate levels.[173]

Lecithin:Cholesterol Acyltransferase

In the circulation, nascent disc-shaped HDL is, under nor- mal conditions, thought to mature into larger spherical HDL. This process entails acquisition of CE in its hydrophilic lipid core, a step made possible through LCAT (**step 4**). The gene is localized in the q21-22 region of chromosome 16 and encodes a 416-amino-acid glycoprotein that is (as apoA-I and ABCA1) expressed in the liver and small intestine where it is secreted into plasma and where it mostly associates with discoidal HDL.[72] This enzyme hydrolyses fatty acids from PC and subsequently transfers and esterifies these to the free hydroxyl group of FC. The acquisition of CE converts disc-shaped HDL into spherical HDL that are predominant in human plasma.[174] Through the esterification of FC, LCAT is thought to maintain a cholesterol gradient that promotes cholesterol efflux from peripheral cells to HDL. The identification of patients with HDL deficiency and abnormal cholesterol and phospholipid tissue deposition have elucidated the fundamental role that LCAT plays in human HDL metabolism.[175,176] LCAT deficiency, in both humans[62] and mice,[177] causes HDL deficiency that is accompanied by accelerated catabolism of apoA-I and apoA-II.[178] Loss of LCAT activity in humans, with 94 LCAT single gene defects reported worldwide, is associated with 2 autosomal-recessive phenotypes, respectively, familial LCAT deficiency exhibiting total loss of enzyme activity and fish-eye disease, a less severe deficient form.[62] Individuals with the former phenotype present with low HDL-C and apoA-I levels, reduced or normal LDL-C levels, accelerated apoA-I/II catabolism, and hypertriglyceridemia, in addition to the typical triad of diffuse corneal opacities, anemia, and proteinuria with renal failure.[179] Patients with fish-eye disease generally only display corneal opacities despite complete HDL deficiency. Studies in homozygote LCAT–deficient mice display severe reductions in apoA-I, HDL-C, and total cholesterol, as in humans with a significant increase in plasma triglycerides,[66,180] whereas heterozygotes have 60% of normal total and HDL-C.[181] Overexpression of human LCAT results in significantly increased HDL-C.[182]

CTP:Phosphocholine Cytidylyltransferase

Jacobs et al[183] showed that CTP:phosphocholine cytidylyl- transferase (encoded by *Pcyt1a,b*)[184] regulates plasma levels of HDL-C and very-LDL (VLDL) in liver-specific cytidylyltransferase alpha (α isoform) KO mice. It concerns a key enzyme in the cytidine diphosphate-choline pathway for the biosynthesis of phosphatidylcholine, a vital component for the structural integrity of mammalian membranes and the primary phospholipid in plasma lipoproteins.[185] Plasma HDL (PC, cholesterol, and apoA-I) was 50% lower in the KO mice than in the control mice, indicating that hepatic PC supply from CTα is vital for plasma HDL.[183] In conclusion, *APOAI, ABCA1,* and *LCAT* are key regulators of HDL metabolism. Remarkably, the loss of a single allele of any of the 3 genes cannot be compensated because all cause similar reductions of HDL-C levels. Apparently, apoAI production, apoAI lipidation, and CE acquisition by nascent HDL are equally important to steady-state levels of plasma HDL-C. The roles of *ApoM* and particularly *PCYT1* in the biogenesis of HDL have primarily been studied in mice, and there are, to our knowledge, no reports on the effects of variation in these genes on human metabolism to date.

Remodelling of HDL in the Circulation

In the circulation, several proteins and enzymes modulate HDL. In humans, these include *CETP*, phospholipid transfer protein (*PLTP*), *LIPC*, endothelial lipase (*LIPG*), and secreted phospholipase A2 (*sPLA2*).[186] Mice lack CETP and sPLA2. Loss-of-function mutations in these genes can underlie either hyperalphalipoproteinemia (*CETP, LIPG, sPLA2*) or hypoalphalipoproteinemia (*PLTP*). Whereas CETP and PLTP are lipid transfer proteins without catalytic activity, the remaining players discussed in this section all exert enzymatic, that is, lipolytic functions that are thought to affect apoAI turnover.

Cholesteryl Ester Transfer Protein

This protein accommodates the transfer of CE from HDL to apoB-containing lipoproteins in exchange for triglycerides (**step 5**). Once CEs are conveyed to apoB-containing lipoproteins, they are made available for uptake of LDL via hepatic receptors.[72] The evidence that CETP is essential for human HDL metabolism came about with the discovery of human CETP deficiency,[137,187]

with 2-fold to 3-fold increases of HDL-C levels and remarkably large HDL. Heterozygous CETP deficiency results in less significant increases in HDL-C levels ranging between 10% and 35%.[105,188] To date, 39 *CETP* (16q21) variants have been reported with most data retrieved from Japanese families. Despite the presence of frequent *CETP* variants with significant effects on HDL-C, the role of CETP in atherogenesis remains controversial.[52,53,189] In mice, which naturally lack CETP, the introduction of the human *CETP* transgene decreases HDL-C and apoA-I levels,[190] whereas overexpression can either increase or decrease atherosclerosis, depending on the introduction of other human genes.[57,191]

Phospholipid Transfer Protein

PLTP is crucial to HDL particle remodelling. As shown in **step 6**, PLTP facilitates the transfer of PL from TRL to HDL with the formation of both larger and smaller particles,[192] whereas it can also induce fusion of smaller HDL.[193] PLTP KO mice show decreased HDL-C and apoAI levels.[90,91] The role of PLTP in the transfer and exchange of PL between TRL and HDL has also been tested in animals overexpressing human PLTP. A 29% increase of PLTP activity promoted net phospholipid movement into HDL and, as a result, HDL phospholipid and FC were significantly increased.[194] Thus far, studies of PLTP (20q12-q13.1) in humans are restricted to association studies showing that variation in the *PLTP* gene is associated with HDL-C levels[89,195] but no cases of human PLTP deficiency have been described.

Hepatic Lipase

Located on chromosome 15q21, this gene encoding hepatic lipase (HL) is involved in breaking-down HDL-TG and PL, thereby reducing HDL size and enhancing the dissociation of lipid-free/lipid-poor apoA-I from larger HDL (**step 7**).[196] Anchored to cell surface proteoglycans in humans (while circulating in mice), HL also has a bridging function promoting receptor-mediated uptake of lipoproteins.[197] Complete *LIPC* deficiency constitutes a rare metabolic condition genetically transmitted in an autosomal recessive pattern, resulting in in- creased HDL-C levels attributable to decelerated HDL catabolism.[139,198,199] To date, ≈60 individuals (8 homozygotes) have been reported worldwide. All affected individuals present with increased plasma cholesterol (>90th percentile) and TG levels and accumulation of large

triglyceride-rich HDL and LDL particles. HL has been proposed to be both proatherogenic and antiatherogenic after studies in mice. Subjects with absent HL activity have been shown to have premature CAD.[196]

Endothelial Lipase

This gene on chromosome 18 encodes for endothelial lipase (EL) a second lypolytic enzyme. It is expressed in the liver, lung, kidney, and placenta. The enzyme has shown to exhibit more phospholipase activity than TG lipase activity with a major preference for HDL instead of TRL (**step 8**). It was first described in 1999 through in vitro expression studies in cells of human origin and through in vivo injection of adenovirus encoding human EL in mice.[82,200] Overexpression of *LIPG* in mice leads to a reduction of HDL-C[79,82] and apoA-I levels. In contrast, loss of EL in mice leads to significant increase in plasma HDL-C[79,201] and reduced atherosclerosis.[80] Like HL, EL has also been shown to be capable of bridging HDL and other lipoproteins with cell surface proteoglycans.[202] An association between *LIPG* variation and HDL-C levels has been confirmed through GWAS,[77,203] and several studies suggest that mechanisms underlying the associations between the *LIPG* SNPs and HDL metabolism may involve loss of function[204] as well as impaired secretion of EL, both resulting in elevated levels of HDL-C.[205] Singaraja et al[206] identified and functionally characterized several partial and complete loss- of-function *LIPG* mutations. Their impact on HDL-C is directly related to their effect on loss of EL function, supporting the hypothesis that antagonism of EL function would provide cardio-protection.[206]

Secreted Phospholipase A2

Encoding for the *sPLA2* is highly expressed in the liver, particularly during acute and chronic inflammatory states.[207] This enzyme hydrolyzes the sn-2 ester bond of phospholipids to release a lysophospholipid and a nonesterified free fatty acid. Overexpression of human group IIa sPLA2[208] in mice (naturally sPLA2-IIA deficient) results in a reduction of HDL-C levels, HDL size, and increased HDL catabolism.[209] Webb et al[208] recently showed that sPLA2-IIa can contribute to atherosclerotic lesion development in mice through a mechanism that is independent of systemic lipoprotein metabolism. Recently, 2 sPLA2-IIA noncoding SNPs have been shown to be functional, making them valuable tools to assess whether the relationship

between sPLA2-IIA and coronary heart dis-ease is causal.[210] To our knowledge, there are no reports on mutations in *sPLA2* in humans. The genes discussed in this section all markedly affect HDL-C levels either through facilitating the transfer of neutral and phospholipids (CETP and PLTP, respectively) between HDL (and among HDL) and apoB-containing lipoproteins or by lipolysis of HDL phospholipids and triglycerides (EL and HL, respectively). The combined local or systemic actions of these factors and those already discussed, however, do not ultimately determine the actual level of HDL-C in plasma. In this regard, it may be noted that all reports discussed to date have merely studied HDL and other lipids under fasting conditions, whereas for a large portion of the human population worldwide this has become a scarce situation. We will continue with studies describing how the catabolism of TRL affects HDL and HDL-C, although these data are, again, mainly obtained after fasting.

Interaction of HDL With TRLs

This section focuses on proteins and enzymes that affect HDL metabolism through their impact on plasma triglyceride lipolysis. These mostly affect the activity of lipoprotein lipase (LPL), the sole enzyme capable of hydrolyzing plasma triglycerides in plasma TRL.[196,211] LPL is synthesized and secreted by parenchymal cells in metabolically active muscle and adipose tissue. At these sites, surface lipid (FC and PL) and apolipoproteins resulting from TRL hydrolysis are conveyed from TRL to HDL (**step 9**).[196]

Lipoprotein Lipase

The *LPL* gene is located on chromosome 8p22 and >160 mutations have been reported. LPL deficiency is an autosomal-recessive disorder characterized by severe hypertriglyceridemia (because of the accumulation of chylomicrons) and marked decreases of HDL-C and LDL-C levels.[32,212] Although homozygote patients can present with severe pancreatitis, heterozygotes do not have clinical complications and show normal to elevated triglyceride levels and decreased HDL-C. LPL KO mice display hypertriglyceridemia and low HDL-C levels, whereas overexpression of LPL causes an increase in HDL-C levels.[86] Several common coding SNPs in the *LPL* gene have been reported to have a significant impact on HDL-C levels,[32] and these associations are confirmed by meta-analysis and are consistent with findings from recent GWAS.[83,213]

Determinants of LPL Activity

Apolipoprotein CII

For its catalytic activity, LPL needs apoC-II as cofactor, a small protein of 79 amino acids present on TRLs and HDL. Human *APOCII* deficiency (20 kindreds reported worldwide) is like LPL deficiency associated with chylomicronemia and low HDL-C.[1,214] All defects in APOCII (19q13.2) concern nonsense mutations. Heterozygote individuals usually present with normal plasma triglyceride levels.[215] In *APOCII*-deficient patients, the mature HDL subfractions have been reported to be reduced or lacking.[216,217]

Apolipoprotein AV

ApoA-V can be considered as a modulator of LPL activity. *APOAV* (11q23) is expressed in the liver and the protein is secreted into plasma, where it associates with VLDL, chylomicrons, and HDL.[218] It seems to be a key modulator of plasma TG homeostasis but the molecular mechanisms are not fully understood.[33,34] ApoA-V may act by increasing LPL activity in a fashion similar to that of apoC-II,[219] although other studies do not support this.[33] Individuals with complete apoA-V deficiency may present with hypertriglyceridemia and low HDL-C, but the penetrance often depends on other deleterious parameters. Heterozygote individuals have normal or moderately elevated plasma TG.[35] Remarkably, *APOAV* gene polymorphisms display the most significant associations with HDL-C levels when compared with genes encoding for other apolipoproteins.[33,34,36] It may be noted, however, that *APOAV* is part of the *AI-CIII-AIV* gene cluster that is highly polymorphic, and genetic variation may also affect the transcription of these genes. Accordingly, this gene cluster is significantly associated with both triglyceride and HDL-C levels in recent GWAS.[78] Of note, in this regard GWAS have identified *APOAI* as a gene with TG as main lipid trait.[61]

GPI-Anchored HDL-Binding Protein-1

The *GPIHBP1* is located on chromosome 8q24.3 and encodes the glycosylphosphatidylinositol (GPI)-anchored HDL-binding protein-1 and was originally identified as an HDL-binding protein,[220] but the finding that *Gpihbp1* knockout mice have severe hypertriglyceridemia revealed an essential role for the protein in the action of LPL in capillary endothelium.[221] In these mice, the

majority of the triglycerides and cholesterol are present in large lipoproteins, whereas HDL-C levels are low. Gpihbp1 is produced in cardiac muscle, skeletal muscle, and adipose tis- sue, and has been suggested to facilitate LPL trafficking over the endothelium and to operate as a scaffold for LPL and its substrates at the luminal side of these cells.[221,222] To date, a few point mutations and 1 large deletion in *GPIHBP1* have been reported in patients who present with severe hypertriglyceridemia[223–225] and low HDL-C.[226]

Inhibitors of the Catalytic Activity of LPL

The LPL reaction is regulated in a spatiotemporal fashion by several inhibitory factors encoded by *APOCIII*, angiopoietin-like 3 (*ANGPTL3*), and *ANGPTL4,* which all affect HDL metabolism.

Apolipoprotein CIII

APOCIII secreted from the liver and, to a lesser extent, by the intestine is a component of both HDL and TRL. Loss-of- function mutations have been associated with higher levels of HDL-C and lower levels of LDL-C and TGs. To date, 12 mutations have been described in *APOCIII* (11q23.3) associated with apparent cardio-protection.[43] Overexpression of human apoC-III in mice results in hypertriglyceridemia,[227] whereas targeted disruption of *Apoc3* results in a reduction of plasma triglyceride and protection from postprandial hypertriglyceridemia.[228] It has also been suggested that apoC-III increases the catabolism of HDL and is involved in other relevant lipid metabolic functions.[43]

GALNT2

UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl-transferase 2 (GalNAc-T2) encoding the polypeptide N-acetylgalactosaminyltransferase 2 has been reported to affect HDL and TG metabolism through glycosylation of apoC-III.[60] This enzyme is involved in the regulation of the O-linked glycosylation of proteins.[229] Common SNPs in this gene through GWAS were shown to be associated with HDL-C and TG levels. Overexpression in mouse liver reduces HDL-C levels, whereas silencing hepatic gene expression leads to an increased HDL-C.[61] In 2 families, it was reported that a functional GALNT2 mutation affects

HDL metabolism by accelerating postprandial TG clearance.[60]

Angiopoietin-Like 3 and Angiopoietin-Like 4

Although *ANGPTL3* (1p31.3) is secreted exclusively from the liver, *ANGPTL4* (19p13.2) is primarily found in those tis- sues that also express LPL. Both encoded proteins can act as inhibitors of LPL activity by promoting, in different ways, the dissociation of the active LPL homodimer into inactive monomers. Angptl4 KO mice exhibit increased LPL activity, 65% to 90% lower TG levels, slightly lower total cholesterol levels, lower HDL-C, and circulating VLDL,[230,231] whereas transgenic mice have reduced post heparin plasma LPL activity and elevated plasma triglycerides.[231] Angptl3 KO mice also show lower plasma triglyceride and cholesterol levels.[232] Interestingly, these mice display a counterintuitive 50% reduction in plasma levels of HDL-C. This has been explained by evidence that *ANGPTL3* also inhibits EL. Thus, a resulting increase of EL activity would reduce plasma levels of HDL-C. Angptl3-deficient mice showed a significant decrease in HDL-PLs and cholesterol, which could be restored through reintroducing ANGPTL3.[233,234]

Double *Angptl3/Angptl4* KO mice die before birth or 2 months after showing almost undetectable low cholesterol and TG levels, therefore proving the pivotal role of *Angptl3/4* in lipoprotein metabolism.[232] Recently, it was shown that rare ANGPTL3,4 gene variants are associated with low plasma TG levels and increased HDL-C in humans.[235,236] All mutant alleles that were associated with low plasma TG levels interfered either with the synthesis or secretion of the protein or with the ability of the ANGPTL protein to inhibit LPL. In contrast to the mouse studies, loss-of-function mutations in *ANGPTL3* in humans were not associated with a decrease in plasma levels of HDL-C.[236] On the contrary, Musunuru et al[237] found that complete *ANGPTL3* deficiency in humans results in extremely low plasma levels of LDL-C, HDL-C, and TG.

Other Modulators of HDL and TG Metabolism

TRIB1 and glucuronic acid epimerase (*GLCE*) have also been shown to affect HDL metabolism in both human and mouse studies.

Tribbles Homolog 1

Tribbles homolog 1 is a member of the recently identified tribbles protein family, mapping within 8q24 locus and with suggested function as adaptor or scaffold protein.[238] Minor alleles in *TRIB1* SNPs have been found to be associated with lower TG, LDL-C, and higher HDL-C, and also with a significantly reduced risk of CAD.[61] Studies in the general population highlighted the strong association between *TRIB1* variation and HDL-C and a less strong, but still significant, association with TG. The mechanism by which *TRIB1* affects lipid metabolism is unknown. It may be mediated through mitogen-activated protein kinase pathway, which is directly controlled by *TRIB1*.[239] Burkhardt et al[240] provided evidence that TRIB1 is implicated in regulation of hepatic lipogenesis and VLDL production in mice: hepatic-specific overexpression of *Trib1* reduces levels of plasma TG and VLDL, LDL-C, and HDL-C by decreasing VLDL production. Conversely, *Trib1*-KO mice showed elevated levels of plasma TG, VLDL, and LDL-C because of increased VLDL production, whereas HDL-C was not significantly affected. These *TRIB1* studies illustrate that HDL-C is not always inversely related to TG levels in the circulation.

GLCE

GLCE is another genetic locus in the 15q21–23 region (which includes *LIPC)*, which was recently linked to HDL-C levels in Turkish families. The gene encodes a glucuronic acid epimerase and is critically important for the biosynthesis of heparin-sulfate proteoglycan, which in turn plays a major role in clearing TRLs from the plasma[241] along with apoE.[242] Moreover, analyses of plasma lipids in $Glce^{+/-}$ mice on the $Apoe^{-/-}$ back- ground support the involvement of *Glce* in lipid metabolism.[243] From this section, it is clear that many factors impact HDL metabolism either through directly affecting the hydrolysis of plasma triglycerides or through modulating hepatic VLDL secretion. These observations seem to have thus far received little attention in the HDL and TG research fields, which may need to change when considering, for example, diabetic dyslipidemia characterized by decreased HDL-C and increased plasma TG.

HDL and Cellular Cholesterol Homeostasis

HDL is known for its important role in acting as an acceptor of cellular cholesterol in which ≥3 major genes encoding ABCA1,[244] ATP-binding cassette transporter G1 (ABCG1),[21] and scavenger receptor class B type 1 (SR-B1)[245] play key roles. This is discussed in detail in another review in this series. The genetics of ABCAI have already been addressed, and this section we address studies of defects in *ABCG1, SR-B1, ORPs* (oxysterol-binding protein–related proteins), and lysosomal storage disorders that affect plasma HDL-C levels.

ATP-Binding Cassette Transporter G1

ABCG1 has been shown to play a fundamental role in the regulation of cellular cholesterol homeostasis through actively mediating cholesterol transport to maturated HDL. The gene is located at 21q22.3, with the highest expression in the macrophages, adrenal glands, heart, lung, and spleen.[246,247] Feeding *ABCG1* KO mice a cholesterol-rich, high-fat diet markedly reduces plasma HDL-C levels and increases biliary cholesterol secretion.[248] Little is known about the role of *ABCG1* in human metabolism. Schou et al[249] reported a functional *ABCG1* promoter variant that associates with increased risk of myocardial infarction and ischemic heart disease in the general population but without affecting levels of HDL-C or other lipids or lipoproteins. Abellán et al,[15] however, described a significant association between promoter variant and HDL-C levels. More recently, interactions of *ABCG1* gene variants with diet were proposed.[16]

Scavenger Receptor Class B Member 1

The *SCARB1* gene (12q24.31) encoding for the main HDL receptor is expressed mainly in steroidogenic tissues and the liver, where it controls the selective uptake of CE from HDL.[27] In contrast to ABCA1 and G1, it mediates bidirectional flux of un-CE between cells and HDL.[27,245,250] *SR-B1* KO mice display a 2-fold increase in plasma HDL-C,[98] accelerated atherogenesis, and disruption of cholesterol transport to the liver.[99,251] *SR-B1* overexpression in mice reduces plasma HDL-C levels.[252] In mice, HDL delivers cholesterol to the adrenal gland for steroid production.[253,254] Consistently, mice lacking SR-B1 show an impaired adrenal glucocorticoid stress response.[255] Genetic

association studies in humans show sex-dependent association with HDL-C and LDL-C levels.[256,257] Several rare point mutations in *SR-B1* in patients with high HDL-C levels have been functionally characterized.[95–97] In one case, carriers of a functional mutation displayed augmented HDL-C levels, reduced cholesterol efflux from macrophages, and mild adrenal insufficiency.[95] In a recent study, it was reported that basal, but not stimulated, corticosteroid metabolism is lessened in carriers of individuals with mutations in LCAT or ABCA1, supporting a role for HDL as a cholesterol donor for basal adrenal steroidogenesis in humans.[258]

Oxysterol-Binding Protein–Related Protein 8

Another gene (12q14) that has been shown to play a role in HDL metabolism is *OSBPL8*, a member of the ORPs family that is known to be implicated as intracellular sterol sensors that regulate cellular functions ranging from sterol, sphingolipid, and neutral lipid metabolism to vesicle transport and cell signalling.[259–261] In previous studies, ORP8 has been shown to affect the expression of *ABCA1* and cellular cholesterol efflux,[262] and with ORP8 knockdown leading to several alterations in the cellular lipidome, including increased levels of both FC and CE.[263] Recently, the first Osbpl8 KO mouse was generated, and Osbpl8 deficiency was found to cause a significant elevation of HDL-C, choline phospholipids, and sex-specific alterations of lipid metabolism.[264]

Glucocerebrosidase

Gaucher disease is the most common of the lysosomal storage disorders, characterized by deficiency of the glucocerebrosidase (encoded by *GBA*) and resulting in accumulation of glucocerebroside in macrophages. This cellular metabolic abnormality leads to chronic systemic inflammation and a heterogeneous, multisystemic phenotype including hepato- splenomegaly, skeletal disease, and cytopenia, in addition to an abnormal cholesterol profile (HDL-C <50 mg/dL).[265,266] Type 1 Gaucher disease is the most prevalent form, with >50 mutations reported to date.[157] Interestingly, although carriers of one *GBA* mutation do not exhibit any Gaucher symptoms, significantly lower HDL-c levels have been reported.[265,267]

Lysosomal Acid Lipase

Lysosomal acid lipase, encoded by *LIPA* (10q23.2–q23.3), is a lysosomal enzyme that hydrolyzes CE and TG and is internalized via receptor-mediated endocytosis of plasma lipoproteins. At present, 47 mutations have been reported that are responsible for Wolman disease or cholesteryl ester storage disease, respectively.[157] Wolman disease is a rare recessive disorder caused by homozygous and compound heterozygous mutations that results in complete lysosomal acid lipase deficiency, with massive storage of CE and TG in most tissues, hepatosplenomegaly, adrenal calcification, HDL-C levels, and anemia.[268] Subjects carrying mutations resulting in residual lysosomal acid lipase activity experience development of the less severe phenotype, cholesteryl ester storage disease, characterized by low HDL-C, hyperlipidemia, hepatic fibrosis, and premature atherosclerosis.[269] The mechanism responsible for low plasma HDL-C is currently unknown but is likely attributable to the reduced FC transported to the plasma membrane, which could affect ABCA1-mediated cholesterol efflux from the cell membrane to extracellular acceptors, such as lipid-poor apoA-I particles.[151,270]

HDL Catabolism

SR-B1, as the main high-affinity receptor for HDL, enables the selective uptake of CE from circulating HDL via apoA-I recognition.[271] This occurs, however, without mediating the degradation of HDL, as is the case for LDL. In humans, plasma levels of HDL-C and apoA-I are inversely related to the catabolism of apoA-I,[272] which takes place in the kidney, where lipid-poor apoA-I is initially filtered at the level of the glomerulus and subsequently is catabolized by proximal renal tubular epithelial cells. Chronic kidney disease is associated with marked reductions of plasma HDL-C.[273] However, only little is known about the molecular mechanisms. A protein involved in this process is cubilin (CUBN; 10p12.31), an extracellular protein synthesized by proximal renal tubular cells and expressed at the apical surface.[274] It has the capability of binding HDL and apoA-I with high affinity and interacting with a co-receptor named megalin or LDL-related protein 2 (4q35.1), a member of *LDLR* gene family, which facilitates uptake and degradation of apoA-I.[275] Studies of cubilin deficiency in animals or humans, however, have not shown marked changes in plasma HDL-C or apoA-I levels.[276] It is currently thought that the rate of renal apoA-I catabolism is determined by both apoA-I

lipidation (ABCA1, LCAT) and apoA-I delipidation processes (EL, HL) as described.[151]

CONCLUSIONS

The unravelling of the causes of severe hypoalphalipoproteinemia and hyperalphalipoproteinemia in humans and mice and the use of candidate gene approaches have helped in discovering the major HDL pathways in the past century. These included those relating to the 3 Mendelian disorders of HDL metabolism (*APOAI, ABCA1,* and *LCAT* deficiency). These key findings have helped to develop novel therapeutic intervention methods, some of which are still undergoing study.[126,277] Since 2008, GWAS have subsequently rediscovered the known genes but also have identified many additional candidate genes or genomic regions that are associated with HDL-C levels. Follow-up reports are discussed in this review. GWAS of lipid metabolism have underscored that HDL-C and TG levels in plasma can barely be considered as independent traits. We have discussed mutations (or targeted disruptions) in genes affecting either or both traits in an attempt to provide a complete picture. This review has used the genetic handholds to describe the major players in HDL anabolism and catabolism, for which studies in both humans and mice were considered. In summary, the de novo synthesis of HDL is dependent on 3 major players, respectively, *APOAI, ABCA1,* and *LCAT*, each of which confer severe HDL deficiency in case of a total gene loss. For the generation of pre–β-HDL, roles for *PCYT1*, *ApoM,* and *OSBPL8* are also recognized. HDL is further modulated in the circulation through lipid transfer proteins (*CETP*, *PLTP*) and lipolytic enzymes (encoded by *LIPC, LIPG, sPLA2*) that affect apoA-I turnover, and mutations in these genes all markedly affect HDL-C levels. The genes that have an impact on HDL metabolism through their effect on plasma TG lipolysis in TRL and modulating hepatic VLDL secretion are, respectively, those affecting/stimulating LPL function (*APOCII, APOA-V,* and *GPIHBP1*) or inhibiting LPL (*APOCIII* and *ANGPTL3,4*) and, finally, those for which it is currently not known what the molecular mechanisms are through which they operate (*TRIB1* and *GLCE*). In addition, we describe the roles of other players in the field, including *OSBPL8*, *GBA,* and *LAL*, that affect cellular but also systemic HDL-C homeostasis. Finally, it is recognized that early lipidation of apoA-I and the lipolysis of HDL-TG and HDL-PC are the apparent major determinants of HDL/ apoA-I clearance by the kidney.

PERSPECTIVES

During the past 14 years, HDL gene finding and candidate gene studies have not delivered major breakthroughs that may relate to the notion that there are no other major HDL genes left to be found. This fits with the fact that the molecular defects responsible for extreme HDL-C phenotypes in patients with clear clinical symptoms have, to our knowledge, all been elucidated. Another point is that several studies have now provided evidence that even in cases of extreme hypoalphalipoproteinemia or hyperalphalipoproteinemia in humans, multiple mutations combined can be responsible for these phenotypes. In other words, the HDL-C trait can be polygenic in even these extreme cases. In the respective studies, only the coding regions of a few[59] (≤197, genes[109]) were investigated. As discussed in this review, ≥40 genes are now reported to be significantly associated with plasma levels of HDL-C and this list is likely to grow, as we previously reported.[150] However, the integration of the effects of multiple rare and common gene variants has only just begun. A recent whole-genome sequencing study provided evidence that common DNA variations can explain most of the heritability of HDL-C levels in a general population sample, whereas most of these variants were found in intergenic regions.[110] The question is whether the genetic HDL picture is nearly complete. This is an intriguing question for especially geneticists. For the HDL scientist, it may be interesting to unravel the molecular mechanisms by which (new) candidate genes affect HDL-C. But where does one start? It is evident that, for example, the effect size of genetic variation identified through GWAS on plasma HDL-C levels is not necessarily related to the potential importance of a candidate gene. For instance, variation in the LCAT gene was indicated by GWAS as being associated with HDL-C levels but only when >100 000 individuals were studied, whereas loss of LCAT function results in HDL deficiency. This means that every candidate gene or regulating entities in intergenic regions could be relevant to the field. What complicates matters is that with the advance of genome sequencing, we are faced with hundreds of putatively functional mutations in DNA in each individual. To help prioritizing, new tools to select the most promising mutations for functional genetic studies are much needed. Coexpression analyses[278] and metabolic profiling[279] may give handholds to further dissect HDL metabolism.

Finally, to improve the understanding of how plasma HDL (and HDL-C) and TG relate to atherogenesis, there is, in our opinion, a need to integrate insights from both fields of re- search. It may help in the understanding of the

pathogenesis of diabetic dyslipidemia (as seen in patients with the metabolic syndrome) characterized by high TG levels and low HDL-C. Integrating knowledge obtained through studies under fasting and nonfasting conditions with a focus on the key candidate genes may probably be a first step to take. Maybe this will help us obtain insight into which parameters determine plasma lipid fluxes that will ultimately lead to a better understanding of which pharmaceutical strategy may reduce the risk of CVD.

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PART 1

Novel insights on the regulation of blood lipid levels - Known and newly identified receptors

"Intelligence without ambition is a bird without wings"

(Salvador Dali`)